

_3/18/08

IN THE CLAIMS

1. (Currently Amended) A method for regeneration of cotton via somatic embryogenesis with substantially synchronized development of embryos after short duration inositol starvation, said process comprising the steps of:

(i) cutting from the germinated cotton seedling an explant, selected from the group consisting of cotyledon, hypocotyl, mesocotyl, and mixtures thereof;

(ii) culturing the explant for the purpose of callus induction on a first solid medium, on a culture medium containing glucose as the carbon source supplemented with Gamborg B5 vitamins, 2,4-D, BA and inositol, at a temperature between 23 to 33° C in light intensity of at least 90 $\mu\text{mol}/\text{m}^2/\text{s}$ under a 16 hour photoperiod for a period of 3-5 weeks, to enable a dedifferentiated callus to form from the explant;

(iii) transferring the callus from the first solid medium to a liquid medium comprising a basal medium containing glucose as the carbon source and supplemented with Gamborg B5 vitamins and culturing a suspension generated thereof at a temperature from 23 to 33° C in a reduced light intensity of 20-40 $\mu\text{mol}/\text{m}^2/\text{s}$, under a 16 hour photoperiod for a period of time sufficient to form embryogenic clumps;

(iv) screening the suspension through metal sieves of different pore sizes to select embryogenic cells, clumps, or both and subculturing the callus containing somatic embryos to said basal medium;

(v) subjecting the embryogenic cells, the clumps, the callus, or any combination thereof to inositol deprivation, consequent upon subculturing it to a second basal medium devoid of inositol for 8-12 days and then returning the culture to inositol containing medium to enable somatic embryos to synchronize developmentally;

(vi) transferring the somatic embryos to an embryo germination medium on a